

What is claimed is:

1. An oligonucleotide which selectively hybridizes with
5 a target DNA sequence associated with pathogenic species of *Guignardia*, said oligonucleotide having a sequence selected from the group consisting of AAAAAGCCGCCGACCTACCT (SEQ ID NO: 1) and TAAAAAAAGCCGCCGACCTAC (SEQ ID NO: 8).
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2. An oligonucleotide which selectively hybridizes with non-pathogenic species of *Guignardia*, said oligonucleotide being selected from the group consisting of GCTACAACGCCGAAATGACCTT (SEQ ID NO: 2), GCCGTCGCCAGCACTC (SEQ ID NO: 3), and GCTACAACGCCGAAATGACC (SEQ ID NO: 9).
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3. An oligonucleotide for priming a DNA amplification of a target DNA sequence associated with pathogenic species of *Guignardia*, said oligonucleotide having a sequence selected from the group consisting of AAAAAGCCGCCGACCTACCT (SEQ ID NO: 1) and TAAAAAAAGCCGCCGACCTAC (SEQ ID NO: 8).
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4. An oligonucleotide for priming a DNA amplification of a target DNA sequence associated with non-pathogenic species of *Guignardia*, said oligonucleotide being selected from the group consisting of GCTACAACGCCGAAATGACCTT (SEQ ID NO: 2), GCCGTCGCCAGCACTC (SEQ ID NO: 3), and GCTACAACGCCGAAATGACC (SEQ ID NO: 9).
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5. A method for priming DNA amplification of a target DNA sequence associated with pathogenic species of *Guignardia*, said method comprising:
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- a) obtaining a DNA sample from a fruit suspected of being infected with *Guignardia*;
- b) providing a forward primer selected from the group consisting of SEQ ID NO: 1 and 8;
- 5 c) providing a reverse primer selected from the group consisting of SEQ ID NO: 6 and 11; and
- d) subjecting said DNA sample and said forward and reverse primers to conditions suitable for polymerase chain reaction, amplification of said DNA in the presence of said primers indicating infection with a pathogenic *Guignardia* species.
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6. A method for differentiating pathogenic species of *Guignardia* from non-pathogenic species of *Guignardia*, comprising the steps of:
- a) obtaining a DNA sample from a *Citrus* fruit suspected of being infected with *Guignardia*;
- b) contacting said DNA with a detectably labeled probe which selectively hybridizes with said DNA, said probe having the sequence of SEQ ID NO: said probe having a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 8, AND SEQ ID NO:9; and detecting specific hybridization if any, samples demonstrating hybridization with SEQ ID NO: 1 or SEQ ID NO: 8 being indicative infection of the *Citrus* fruit with pathogenic *Guignardia* and samples demonstrating hybridization with SEQ ID NOS: 2, 3, or 9 being indicative of infection of the *Citrus* fruit with non-pathogenic species of *Guignardia*.
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7. A method for differentiating pathogenic species of *Guignardia* from non-pathogenic species of *Guignardia*, comprising the steps of:
- 5 a) obtaining a DNA sample from a *Citrus* fruit suspected of being infected with *Guignardia*;
- 10 b) immobilizing said DNA sample on a solid support;
- 15 c) probing said immobilized sample DNA with a detectably labeled probe, said probe having a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 8, AND SEQ ID NO:9; and
- 20 d) assessing said solid support for hybridization of said probe to said immobilized DNA, samples demonstrating hybridization with SEQ ID NO: 1 or SEQ ID NO: 8 being indicative infection of the *Citrus* fruit with pathogenic *Guignardia* and samples demonstrating hybridization with SEQ ID NOS: 2, 3, or 9 being indicative of infection of the *Citrus* fruit with non-pathogenic species of *Guignardia*.
8. A method of screening for *Citrus* Black Spot disease, said method comprising
- 25 a) obtaining a nucleic acid sample; and
- 30 b) assaying the nucleic acid sample for the presence of a sequence which hybridizes with SEQ ID NO: 1 or SEQ ID NO: 8 or SEQ ID NO: 4, wherein the presence of said hybridizing sequence is indicative of pathogenic *Guignardia* infection causing *Citrus* Black Spot disease.
9. A method in accordance with claim 5, wherein said assaying further comprises amplification

of the ITS region of *Guignardia* rDNA containing an ITS nucleic acid, said ITS nucleic acid sequence being amplifiable using primers consisting of SEQ ID NO: 12 and SEQ ID NO: 13.

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10. A method in accordance with claim 5, wherein
said polymerase chain reaction is primed by SEQ
ID NOS: 8 and 11.
15. A kit for use in screening for *Citrus Black*
Spot disease comprising a pathogen-specific
oligonucleotide probe immobilized on solid
matrix, and further comprising means for
amplifying a test sample's nucleic acid
encoding all or part of the rDNA gene, wherein
said test sample's nucleic acid comprises a
sequence of SEQ ID NO: 4.
20. A kit in accordance with claim 11, wherein said
amplifying means comprises:
(a) a primer pair of oligonucleotides
comprising a first oligonucleotide having the
sequence of SEQ ID NO: 1 or SEQ ID NO: 8 and a
second oligonucleotide having the sequence of
SEQ ID NO: 6 or SEQ ID NO: 11; and
(b) reagents necessary to perform PCR.
25. A kit in accordance with claim 12, wherein the
oligonucleotide pair comprises SEQ ID NO: 8 and
SEQ ID NO: 11.

14. A kit for use in screening for non-pathogenic *Guignardia* species comprising a non-pathogen-specific oligonucleotide probe immobilized on solid matrix, and further comprising means for amplifying a test sample's nucleic acid encoding all or part of the rDNA gene, wherein said nucleic acid comprises a sequence of SEQ ID NO: 5.
- 10 15. A kit in accordance with claim 14, wherein said amplifying means comprises:
(a) a primer pair of oligonucleotides comprising a first oligonucleotide having the sequence of SEQ ID NO: 2 or SEQ ID NO: 9 and a second oligonucleotide having the sequence of SEQ ID NO: 6 or SEQ ID NO: 10 or SEQ ID NO: 11; and
(b) reagents necessary to perform PCR.
- 15 20. A kit in accordance with claim 15 wherein the oligonucleotide pair comprises SEQ ID NO: 9 and SEQ ID NO: 10.
- 25 30. 35. 17. A method for identifying pathogenic *Guignardia* species in a sample, said method comprising the steps of:
a) culturing *G. citricarpa*;
b) subjecting said cultured *G. citricarpa* to conditions which effect lysing of said *G. citricarpa*, thereby releasing DNA from said hyphae;
c) contacting said released DNA with a primer pair of oligonucleotides comprising a first oligonucleotide having the sequence of SEQ ID

NO: 1 or SEQ ID NO: 8 and a second
oligonucleotide having the sequence of SEQ ID
NO: 6 or SEQ ID NO: 11 under conditions where
amplification of pathogenicity-associated ITS
sequences occurs, if said pathogenic *Guignardia*
is present in said sample; and
d) detecting said amplified sequence, if
present.

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18. A method as claimed in claim 17, wherein said
amplified sequence is detected via
incorporation of a detectable label.

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19. A method as claimed in claim 17, wherein said
amplified sequence is detected by gel
electrophoresis of said amplified sample.

20. A method as claimed in claim 17, wherein said
ITS sequences are amplified using a primer set
having SEQ ID NOS: 12 and 13 prior to
amplification of pathogenicity related
sequences employing a primer set having SEQ ID
NO: 8 and 11.

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21. A method for identification of pathogenic
Guignardia citricarpa, said method comprising:
a) contacting a tissue section containing a
black spot lesion with a permeabilization
agent;
b) contacting said permeabilized lesion DNA
with a detectably labeled oligonucleotide
having a sequence of SEQ ID NO: 1 or SEQ ID NO:
8; and

c) detecting hybridization of said oligonucleotide to said DNA, if any, said hybridization indicating the presence of pathogenic *G. citricarpa*.